bio-protocol Preprint

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Preparation of DCs

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An abbreviated version of this protocol was published in eLIFE in Feb 2021

Gut Helicobacter presentation by multiple dendritic cell subsets enables context-specific regulatory T cell generation

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Detailed protocol

Preparation of Dendritic Cells

Step 1. Remove dMLNs from mice. Dendritic cells were harvested from the dMLNs of ~5-10, 3-5 week old Ly5.1 *Foxp3*$^{IRES\text{-}GFP}$ mice in our colony for *ex vivo* and FACS experiments, and from cDC deficient mice for qPCR experiments.

Step 2. Dissociate dMLNs. 5 dMLNs were pooled per 15 ml conical tube (Thermo Fisher #14-959-53A) containing 10 ml RPMI (Thermo Fisher #SH3025502) with 5% bovine calf serum (Thermo Fisher #SH3062602), 1x penicillin/streptomycin (Cytiva HyClone #SV30010), 1 mM sodium pyruvate (Lonza #BW13-115E), 1x non-essential amino acids (Cytiva HyClone #SH3023801), 50 mM beta-mercaptoethanol (VWR #97064-588), 65.8 mg/ml collagenase VIII (Sigma #C2139), and 0.2 U/ml dispase (Thermo Fisher #CB-40235), for 45 minutes at 37°C with 300 RPM continuous magnetic stirring (stir bar 7.9 mm x 12 mm: VWR #58948-116) until tissue was completely dissociated. Suspensions were filtered through an 80 mm mesh filter (Component Supply #U-CMN-80) into a new 15 ml conical tube and centrifuged at 340 RCF for 5 minutes at 4°C.

Step 3. Block and stain cells. The supernatant was removed, and cells were resuspended in 2.5 ml of "sort buffer" PBS (Cytiva HyClone #SH30028.03) containing 5% bovine calf serum and 10 mg/ml anti-CD16/CD32 (BioXCell #BE0307). Cells were incubated on ice for 20 minutes after which 2.5 ml 2x fluorescently-labeled antibody mix was added to each tube (2.5 ml sort buffer with anti-CD3e, anti-B220, anti-CD19, anti-IAb, anti-CD11c, anti-CD103, anti-CD11b; refer to Key resources table for catalog numbers and dilutions). Cells were incubated on ice, in the dark for 30 minutes. Cells were spun at 340 RCF for 5 minutes and washed with 10 ml sort buffer.

Step 4. Sort cDC subsets. The supernatant was removed, cells were resuspended in 1 ml sort buffer, and cell suspension was filtered through a 40 mm mesh (Component Supply #U-CMN-40) into a sterile 5 ml culture tube with lid (VWR #60818-500). Cells were sorted on a BD FACSAria IIu at no greater than 14,000 events/second into sterile 5 ml culture tubes containing 4 ml complete DMEM (DMEM (Thermo Fisher #SH3008101) with 10% fetal bovine serum (Thermo Fisher #16000044), 1x Glutamax (Thermo Fisher #35050061), 50 mM beta-mercaptoethanol, 1 mM sodium pyruvate, 10 mM HEPES (Thermo Fisher #15630080), 1x non-essential amino acids, and 1x penicillin/streptomycin) using the following markers: migratory cDCs ($CD3e^-$ $B220^-$ $CD19^-$ IAb^{hi} $CD11c^{int}$), resident cDCs ($CD3e^-$ $B220^-$ $CD19^-$ IAb^{int} $CD11c^{hi}$), $CD103^+$ SP ($CD3e^-$ $B220^-$ $CD19^-$ IAb^{hi} $CD11c^{int}$ $CD103^+$ $CD11b^-$), DP ($CD3e^-$ $B220^-$ $CD19^-$ IAb^{hi} $CD11c^{int}$ $CD103^+$ $CD11b^+$), and $CD11b^+$ SP ($CD3e^-$ $B220^-$ $CD19^-$ IAb^{hi} $CD11c^{int}$ $CD103^-$ $CD11b^+$).

Step 5. Prepare cDCs for culture. Sorted cells were spun at 340 RCF for 5 minutes and resuspended in 1 ml of complete DMEM. Cells were allowed to rest on ice for 30 minutes, after which cell concentrations were calculated by hemocytometer for culture.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Russler-Germain, E. and Hsieh, C. (2021). Preparation of DCs. Bio-protocol Preprint. bio-protocol.org/prep1110.
2. Russler-Germain, E. V., Yi, J., Young, S., Nutsch, K., Wong, H. S., Ai, T. L., Chai, J. N., Durai, V., Kaplan, D. H., Germain, R. N., Murphy, K. M. and Hsieh, C.(2021). Gut Helicobacter presentation by multiple dendritic cell subsets enables context-specific regulatory T cell generation. eLIFE. DOI: 10.7554/eLife.54792